

Discriminating Lyme Neuroborreliosis from Other Neuroinflammatory Diseases by Levels of CXCL13 in Cerebrospinal Fluid[†]

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CXCL13 in cerebrospinal fluid (CSF) could be an important component for diagnosing Lyme neuroborreliosis (LNB). Levels of intrathecal CXCL13 were determined for 58 LNB patients and 210 controls; sensitivity was 88% and specificity was 89% (cutoff, 250 pg of CXCL13/ml of CSF). Elevated levels of CXCL13 can aid in the diagnosis of LNB, but levels should be interpreted with care.

Diagnosing Lyme neuroborreliosis (LNB) is difficult because one of the most specific markers, the antibody index (AI), is negative in 21 to 45% of patients (1). Intrathecal levels of CXCL13 have been suggested to be a potential biomarker for LNB. CXCL13 is produced by antigen-presenting cells and is a selective chemoattractant for B cells and B-helper T cells. It has been shown that CXCL13 is expressed at high levels in cerebrospinal fluid (CSF) from LNB patients, while levels were barely detectable in CSF from subjects with noninflammatory neurological disease. Overall sensitivity for LNB ranged from 96 to 100%, and specificity ranged from 63 to 98% (3, 6, 11, 12). Case reports describing early diagnosis of LNB using CXCL13 levels in CSF have already been published (5, 9).

Our aim was to determine the diagnostic potential of levels of intrathecal CXCL13 to distinguish acute and late LNB from other central nervous system diseases in the pediatric and adult population.

Patients were identified retrospectively using the OLVG Hospital laboratory information management system. CSF and serum samples from 58 LNB patients before treatment were included. Criteria for diagnosing LNB patients were that their meningitis had no other cause and that they had three of the following four characteristics: positive serology at presentation, pleocytosis, positive AI with an Ideia Lyme neuroborreliosis kit (Oxoid, Cambridge, United Kingdom), and objective neurological complaints with clinical improvement after treatment. From this group, definite LNB patients ($n = 45$) were those who had a pleocytosis and a positive AI, and probable LNB patients ($n = 13$) were those who had either pleocytosis ($n = 12$) or a positive AI ($n = 1$) (4). Ninety-one percent of the LNB patients presented within 6 months of the start of complaints; the range was 7 days to 48 months. Forty-one percent of LNB patients were children. Most LNB patients presented

with a facial nerve paralysis (60%) or meningoradiculitis (26%). Seventeen percent of LNB patients reported experiencing erythema migrans (EM) before or at presentation.

As controls, we included 36 patients with Lyme borreliosis that did not meet the criteria for LNB, 93 patients with an infectious cause of meningitis/encephalitis, 62 patients with neurological inflammatory diseases, and 12 patients with non-inflammatory neurological complaints. Furthermore, seven HIV patients with no neurological complaints or evidence of an intrathecal infection were tested. For patient characteristics, see Table S1 in the supplemental material.

CSF samples were tested with a Quantikine human CXCL13/BLC/BCA-1 immunoassay (R&D Systems, Minneapolis, MN).

Results of the levels of CXCL13 in CSF are shown in Fig. 1. Median levels of CXCL13 were significantly elevated in LNB patients compared to those in the Lyme nonneuroborreliosis controls (medians, 1,183 and 3 pg of CXCL13/ml of CSF, respectively; $P < 0.001$).

Receiver operating characteristic (ROC) analysis revealed an optimal cutoff of 250 pg/ml, which resulted in 88% sensitivity and 89% specificity (Fig. 2). Results of intrathecal CXCL13 using the cutoff of 250 pg/ml for LNB patients and controls are shown in Table 1. CSF levels of CXCL13 correlated with the amount of intrathecal leukocytes in the CSF at presentation ($R = 0.172$; $P < 0.001$), but the seven LNB samples with CXCL13 levels of <250 pg/ml did not have exceptionally low CSF leukocyte counts (see Fig. S1 in the supplemental material).

Previously, a sensitivity of 96 to 100% was reported for CXCL13 in cases of LNB, but two studies did not define a cutoff (3, 6). One study defined a cutoff for CXCL13 levels expressed as ng of CXCL13/g of total protein in CSF (11). ROC curve analysis for the amount of CXCL13 per milliliter compared to the amount of CXCL13 per gram of total protein showed a similar area under the curve (AUC) in our population (0.91 to 0.90, respectively). Analysis using the cutoff of 337 ng/g in our population led to a decrease in sensitivity and specificity to 82% and 88%, respectively. One study defined a cutoff of 142 pg/ml of CSF. In our study, such a cutoff led to a sensitivity of 90% and a specificity of 84% (12).

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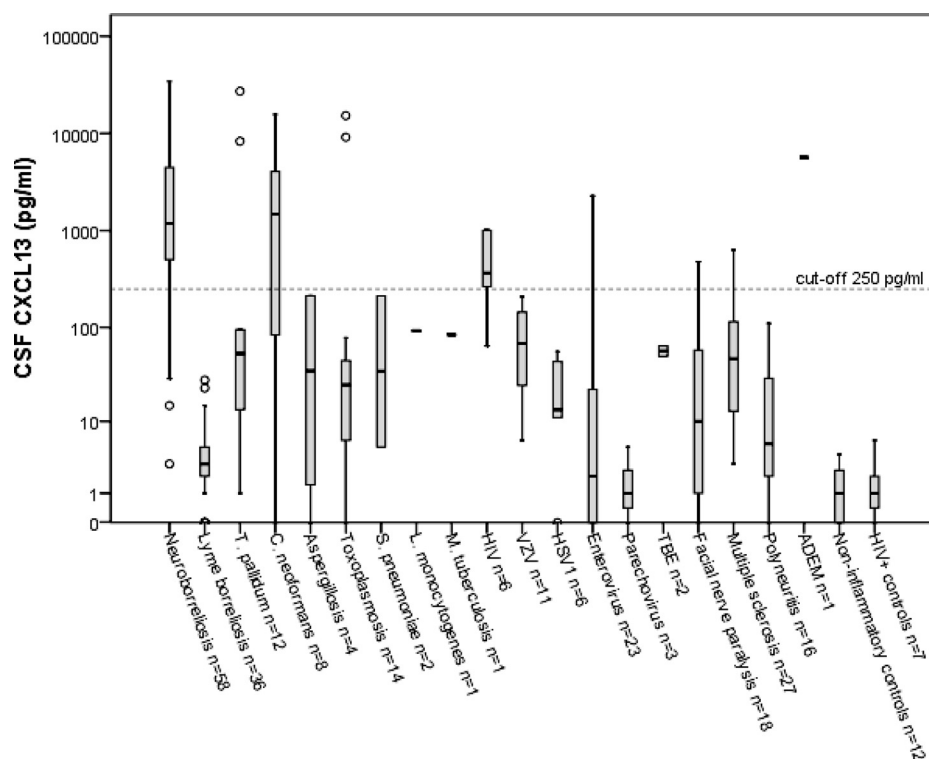


FIG. 1. Levels of CXCL13 in CSF of LNB patients and controls. Horizontal lines indicate medians, bars represent interquartile ranges, lines represent the 95% confidence interval, and open circles represent outliers. The reference line is located at the cutoff of 250 pg/ml as determined by ROC curve analysis for optimal sensitivity and specificity for discriminating LNB patients from controls. T. pallidum, *Treponema pallidum*; C. neoformans, *Cryptococcus neoformans*; S. pneumoniae, *Streptococcus pneumoniae*; L. monocytogenes, *Listeria monocytogenes*; M. tuberculosis, *Mycobacterium tuberculosis*; VZV, varicella-zoster virus; HSV, herpes simplex virus; TBE, tick-borne encephalitis virus.

A possible explanation for the decreased sensitivity might be that storing samples at -20°C before testing could decrease levels of CXCL13. However, the duration of storage of a sample did not result in lower levels of CXCL13 in the

LNB group, as the median value of CXCL13 for up to 5 years of storage did not decrease ($R^2 = 0.015$; $P = 0.4$) (data not shown).

Another explanation for the decreased sensitivity could be

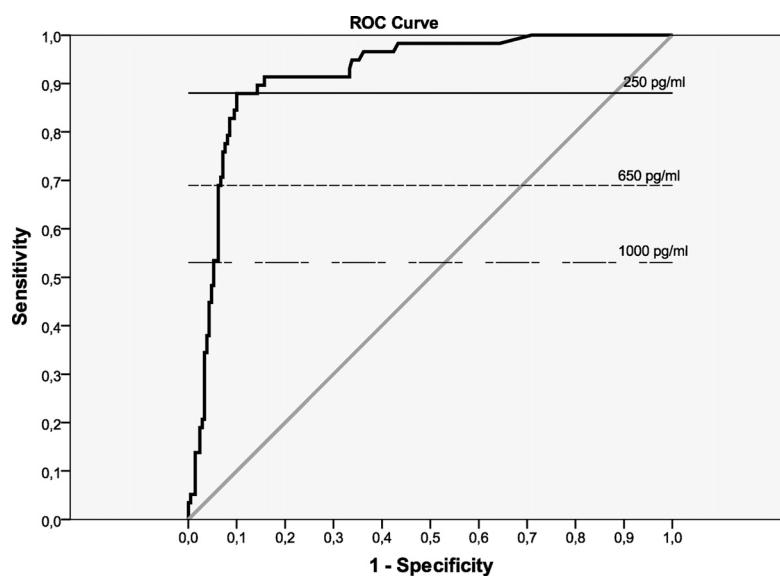


FIG. 2. ROC curve analysis using levels of CXCL13 to discriminate between LNB patients and all controls. Horizontal lines show three different cutoff values for levels of CXCL13. The optimal cutoff was 250 pg/ml.

TABLE 1. Patients and controls positive for intrathecal CXCL13^a

Subject characteristic	No. (%) of LNB patients and controls positive for intrathecal CXCL13 at indicated level (pg/ml)			
	Patients (n = 58)		Controls (n = 210)	
	≥250	<250	≥250	<250
AI positive	42 (72)	4 (7)	0 (0)	4 (2)
AI negative	9 (16)	3 (5)	23 (11)	183 (87)
Pleocytosis	50 (86)	7 (12)	19 (9)	76 (36)
No pleocytosis	1 (2)	0 (0)	4 (2)	111 (53)
Definite LNB	41 (71)	4 (7)		
Probable LNB	10 (17)	3 (5)		
No LNB			23 (11)*	187 (89)

^a Cutoff, 250 pg of CXCL13/ml of CSF. *, no CXCL13-positive controls had serology positive for Lyme.

the different study populations. As is shown in Table 1, the sensitivity was 91% (41/45) in definite LNB cases in which the correct diagnosis was ensured by the presence of pleocytosis in combination with a positive AI. In probable LNB cases, the sensitivity was only 77% (10/13), while in this population, the detection of elevated levels of CXCL13 would be of special interest.

Furthermore, the present study is one of the first to include children. CSF CXCL13 levels were lower in children than in adults with LNB, but this difference was not significant (medians, 932 pg/ml and 1,678 pg/ml, respectively; $P = 0.4$). Moreover, there were not more children with a CXCL13 of <250 pg/ml than there were adults with these levels.

Eleven percent of the controls had CXCL13 levels of over 250 pg/ml CSF (Table 1). These have been specified in Table 2. We identified several infectious diseases, for instance, previously unstudied *Cryptococcus neoformans* meningitis, that can cause highly elevated levels of CXCL13 in the CSF. Previous studies also reported that some bacterial and viral cases of meningitis resulted in moderate elevation of intrathecal CXCL13 (3, 11).

Some immunocompromised patients had elevated expression of CXCL13 intrathecally. One patient with hypogammaglobulinemia had elevated levels of CXCL13. The specificity of the assay is lower in the HIV-positive (HIV⁺) controls; overall specificity in the HIV-negative (HIV⁻) population was 92% (153/166), while the specificity in the HIV⁺ controls with a neuroinflammatory disease was only 77% (34/44). The group of HIV⁺ patients without signs of intrathecal inflammation did not show elevated CXCL13 levels, indicating that the elevated levels of CXCL13 are present only during an infection of the central nervous system. Elevated levels of CXCL13 in the blood of HIV-positive patients already have been described and are associated with altered expression of the CXCL13 receptor (CXCR5), causing B-cell dysfunction (2, 13). HIV infection should be excluded in individuals with elevated levels of CXCL13 in CSF.

Patients with autoimmune diseases and elevated expression of CXCL13 intrathecally have also been described previously; patients with multiple sclerosis (MS) have shown elevated CXCL13 levels (6, 10). We confirm this finding and additionally find that patients with acute disseminated encephalomy-

TABLE 2. High CXCL13 levels in controls^a

Diagnosis of control subject	No. of subjects with high CXCL13 levels/total no. of subjects		
	Total	HIV ⁺	HIV ⁻
<i>Treponema pallidum</i> infection	2/12	2/8	0/4
<i>C. neoformans</i> infection	6/8	2/4	4/4
Toxoplasma encephalitis	2/14	0/12	2/2*
HIV meningitis	5/6	5/6	
Enterovirus meningitis	1/23	0/1	1/22**
Facial nerve paralysis	1/18	1/5	0/13
Multiple sclerosis	5/27	0/0	5/27
ADEM	1/1	0/0	1/1

^a High CXCL13 levels are those ≥250 pg of CXCL13/ml of CSF. *, both of these subjects were congenital toxoplasmosis patients; **, the subject with an elevated level was suffering from hypogammaglobulinemia.

elitis (ADEM) and patients developing Henoch-Schönlein purpura (HSP) after an enteroviral infection can have high levels of CXCL13. HSP and systemic lupus erythematosus (SLE) share common clinical features, and for SLE, elevated blood CXCL13 levels have already been described (7, 8). We conclude that CXCL13 levels in CSF can also be elevated in patients with autoimmune diseases.

Treatment of LNB leads to a vast reduction in CXCL13 CSF levels, which makes CXCL13 a potential marker for studying disease activity and effective clearance after treatment (3, 11). In our study, follow-up CXCL13 levels in the CSF of four LNB patients were determined 30 to 350 days after adequate treatment. In all four patients, levels of CXCL13 declined after treatment to a value below 40% of the initial value (data not shown).

In conclusion, high levels of intrathecal CXCL13 expression are found in most, but not all, adult and pediatric patients with LNB. Some immunocompromised patients and patients with an autoimmune disorder which can have a presentation clinically similar to that for LNB have high levels of CXCL13 in their CSF. Determining levels of CXCL13 as a marker for LNB can aid in the diagnosis but should be interpreted with care.

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